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Dated 29th December 1992

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The Patent Office

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Form 1/77

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1 Please give the title of the invention: PRODUCTION OF RECOMBINANT CHIMERIC PROTEINS FOR VACCINE USE

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Country *(and State of incorporation, if appropriate)*: Canada

2b If you are applying as an individual or one of a partnership please give in full:

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PRODUCTION OF RECOMBINANT CHIMERIC PROTEINS FOR VACCINE USE

The present invention relates to the engineering and expression of chimeric genes, particularly those containing sequences from the genes coding for the major immunogenic proteins of both human Parainfluenza virus (PIV) and Respiratory syncytial virus (RSV). The present invention also relates to the formulation of various recombinant PIV/RSV immunogens to produce safe and efficacious vaccines capable of protecting infants and young children against infection with both PIV and RSV.

Human Parainfluenza virus types 1, 2, 3 and Respiratory syncytial virus types A and B are the major viral pathogens responsible for causing severe respiratory tract infections in infants and young children. Safe and effective vaccines for protecting infants against these viral infections are not available and are urgently required. It is anticipated that the development of a single recombinant immunogen capable of simultaneously protecting infants against infection with both Parainfluenza and Respiratory syncytial viruses could significantly reduce the morbidity and mortality caused by these viral infections.

Identification of the major immunogenic proteins of RSV and PIV has provided the scientific basis for designing the chimeric RSV/PIV immunogens described herein. It has been reported that a protective response is contingent on the induction of neutralizing antibodies against the major viral surface glycoproteins. For PIV, these protective immunogens are the HN protein which possesses both hemagglutination and neuraminidase activities and the fusion (F) protein, which is responsible for both fusion of the virus to the host cell membrane and cell-to-cell spread of the virus. For RSV, the two major immunogenic proteins are the 80-90 kDa G glycoprotein and the 70 kDa fusion (F) protein.

The G and F proteins are thought to be functionally analogous to the PIV HN and F proteins, respectively.

In accordance with the present invention, the inventors have specifically engineered several model PIV/RSV chimeric genes containing relevant sequences from selected genes coding for the PIV-3 and RSV surface glycoproteins. All genes in the chimeric constructs were obtained from recent clinical isolates of PIV-3 and RSV. The chimeric gene constructs include gene sequences from either PIV-3 F or HN genes linked to either RSV G (subtypes A and B) or F genes in all possible relative orientations and combinations.

The constructs may consist of either the entire gene sequences or gene segments coding for immunogenic epitopes thereof. In addition, the present invention also includes trimeric gene constructs containing the PIV and RSV genes or gene segments linked in all possible relative orientations. For example:

F_{PIV} - HN_{PIV} - F or G_{RSV}

F_{PIV} - F_{RSV} - G_{RSV}

HN_{PIV} - F_{RSV} - G_{RSV}

The chimeric and trimeric genes are sub-cloned into appropriate vectors for expression in both mammalian and insect cells. Alternatively, recombinant poxviruses and transformed mycobacteria (BCG) can be used for immunization. Chimeric PIV/RSV proteins present in either the supernatants or cell lysates of transfected cells then are purified by a combination of conventional chromatographic procedures. To evaluate the immunogenicity and protective ability of the combinant proteins, guinea pigs, hamsters and cotton rats are immunized with either recombinant BCG or poxviruses or with varying doses of the purified chimeric PIV/RSV proteins administered in the presence of an appropriate adjuvant, such as aluminum phosphate. In an attempt to further enhance the immunoprotective ability of the

chimeric proteins, the recombinant antigen may contain or be supplemented with other immunogenic proteins of PIV and RSV produced either by genetic engineering techniques or purified from the virus by a series of chromatographic procedures. The final vaccine preparation, when formulated with aluminum phosphate as an adjuvant, can be used as a readily injectable preparation for protecting humans against infection with both PIV-3 and RSV. The invention also includes the use of delivery systems, such as iscoms and liposomes, as well as adjuvants other than aluminum phosphate. The effectiveness of the invention is not limited to the preparation of recombinant chimeric PIV-3 and RSV proteins, but is applicable to the production of chimeric immunogens composed of either the entire sequences or regions of the major immunogenic regions from other Paramyxoviruses linked in tandem.

EXAMPLES

Methods for cloning and sequencing the PIV-3 and RSV genes as well as the procedures for sub-cloning the genes into appropriate vectors and expressing the gene constructs in mammalian and insect cells are not explicitly described in this disclosure but are well within the scope of those skilled in the art. The drawings which accompany and form part of this specification are referred to in the Examples.

Example 1:

This Example outlines the strategy used to clone and sequence the PIV-3 F, HN and RSV F genes. These genes were used in the construction of the F_{PIV-3}-F_{RSV} and F_{RSV}-HN_{PIV-3} chimeric genes detailed in Examples 2 to 4 and Example 8, respectively.

Two PIV-3 F gene clones were obtained from cDNA derived from viral RNA extracted from a recent clinical isolate of PIV-3. The PIV-3 HN and RSV F genes were cloned from a cDNA library prepared from mRNA isolated

from MRC-5 cells infected with clinical isolates of either PIV-3 or RSV. The PIV-3 F, HN and RSV F gene clones were sequenced by the dideoxynucleotide chain termination procedure. Sequencing of both strands of the genes was performed by a combination of manual and automated sequencing.

The nucleotide and amino acid sequences of the PIV-3 F gene is presented in Figure 1 and the restriction map of the gene is outlined in Figure 2. Sequence analysis of the 1844 nucleotides of two PCR amplified PIV-3 F gene clones confirmed that the clones were identical. Comparison of the coding sequence of the PCR-amplified PIV-3 F gene clone with that of the published PIV-3 F gene sequence revealed a 2.6% divergence in the coding sequence between the two genes resulting in 14 amino acid substitutions.

Figure 3 shows the nucleotide and amino acid sequences of the PIV-3 HN gene and the restriction map of the gene is presented in Figure 4. Analysis of the 1833 nucleotide sequence from two non-PCR amplified HN clones confirmed that the sequences were identical. A 4.4% divergence in the coding sequence of the PIV-3 HN gene was noted when the sequence was compared to the published PIV-3 HN coding sequence. This divergence resulted in 17 amino acid substitutions in the coding sequence of the non-PCR amplified PIV-3 HN gene.

The nucleotide and amino acid sequences of the RSV F gene is reported in Figure 5 and the restriction map of the gene is shown in Figure 6. Analysis of the 1859 nucleotide sequence from two RSV F clones verified complete sequence homology between the two clones. Comparison of this nucleotide sequence with that reported for the RSV F gene revealed approximately 1.8% divergence in the coding sequence resulting in 11 amino acid substitutions.

The full-length PIV-3 F, HN and RSV F genes were

cloned into the multiple cloning site of a Bluescript-based vector either by blunt end ligation or using appropriate linkers. The cloning vectors containing the PIV-3 F, HN and RSV F genes were named pPIVF, pPIVHN and pRSVF, respectively.

Example 2:

This Example illustrates the construction of a Bluescript-based expression vector containing the chimeric F_{PIV-3} -F_{RSV}. This chimeric gene construct contained the 5'-untranslated region of the PIV-3 F gene but lacked the hydrophobic anchor and cytoplasmic domains of both the PIV-3 and RSV F genes.

To prepare the PIV-3 portion of the chimeric gene, the full-length PIV-3 gene lacking the transmembrane coding region and cytoplasmic tail was retrieved from plasmid pPIVF by cutting the polylinker with EcoRV and the gene with BsrI. A BsrI-BamHI oligonucleotide cassette (Fig. 7A) containing a PpuMI site and three successive translational stop codons was ligated to the truncated 1.6 Kb EcoRV-BsrI PIV-3 F gene fragment and cloned into the EcoRV-BamHI sites of a bluescript based-expression vector containing the human methallothionein promoter and the poly A and IVS sequences of the SV40 genome to generate plasmid pME1.

To engineer the RSV F gene component of the chimeric construct, the RSV F gene lacking the transmembrane coding region and cytoplasmic tail was retrieved from plasmid pRSVF by cutting the polylinker with EcoRI and the gene with BspHI. A synthetic BspHI-BamHI oligonucleotide cassette (Fig. 7B) containing three successive translational stop codons was ligated to the 1.6 Kb truncated RSV F gene and cloned into the EcoRI-BamHI sites of the Bluescript-based expression vector to produce plasmid ES13A. Plasmid ES13A was then cut with EcoRI and PpuMI to remove the leader and F2 coding sequences from the truncated RSV F gene. The

leader sequence was reconstructed using an EcoRI-Ppu oligocassette (Fig. 7C) and ligated to the RSV F1 gene segment to generate plasmid ES23A.

To prepare the chimeric F_{PIV-3}-F_{RSV} gene, containing the 5'-untranslated region of the PIV-3 F gene linked to the truncated RSV F1 gene fragment, plasmid pME1 (containing the 1.6 Kb truncated PIV-3 F gene) was first cut with PpuMI and BamHI. The 6.2 Kb PpuMI-BamHI restricted pME1 vector was dephosphorylated with intestinal alkaline phosphatase. The 1.1 Kb RSV F1 gene fragment was retrieved from plasmid ES23A by cutting the plasmid with PpuMI and BamHI. The 1.1 Kb PpuMI-BamHI RSV F1 gene fragment was cloned into the PpuMI-BamHI sites of the dephosphorylated pME1 vector to generate plasmid ES29A. This chimeric gene construct contained the 5'-untranslated region of the PIV-3 f gene but lacked the hydrophobic anchor domains and cytoplasmic tails of both the PIV-3 and RSV F genes.

Example 3:

This Example illustrates the construction of a Bluescript-based expression vector containing the PIV-3 F gene lacking both the 5'-untranslated and transmembrane anchor regions.

Plasmid pPIVF containing the full length PIV-3 F gene was cut with BamHI, blunt ended with Klenow polymerase and then cut with BsrI to remove the transmembrane coding region and cytoplasmic tail. The Bluescript-based expression vector (containing the human methallothionein promoter and poly A and IVS sequences of the SV40 genome) was cut with SmaI and BamHI. A synthetic BsrI-BamHI oligonucleotide cassette (Fig. 7D) containing a translational stop codon was ligated with the 1.6 Kb blunt ended-BsrI PIV-3 F gene fragment to the 4.5 Kb SmaI (blunt ended)-BamHI restricted expression vector to produce plasmid pMpFB. The PIV-3 F gene of this construct lacked the transmembrane coding region

but contained the 5′-untranslated region. To engineer a plasmid containing the PIV-3 F gene devoid of both the 5′-untranslated region and the hydrophobic anchor domain, plasmid pMpFB was cut with EcoRI and BstBI. An EcoRI-BstBI oligocassette (Fig. 7E) containing the sequences to reconstruct the signal peptide and coding sequences removed by the EcoRI-BstBI cut was ligated to the 6.4 Kb EcoRI-BstBI restricted pMpFB vector to produce plasmid pMpFA. The PIV-3 F gene of this construct lacked both the 5′-untranslated region and the 3′-transmembrane anchor domain.

Example 4:

This Example illustrates the construction of the chimeric F_{PIV-3}-F_{RSV} gene composed of the truncated PIV-3 F gene devoid of the 5′-untranslated region linked to the truncated RSV F1 gene.

To prepare this chimeric gene construct, plasmid ES29A (Example 2) was cut with BstBI and BamHI to release the 2.4 Kb BstBI-BamHI PIV-3 F2 + 1-RSV F1 chimeric gene fragment. This BstBI-BamHI chimeric gene fragment was isolated from a low melting point agarose gel and cloned into the BstBI-BamHI sites of the dephosphorylated vector pMpFA to produce plasmid ES60A. This construct contained the PIV-3 F gene lacking both the 5′-untranslated region and the hydrophobic anchor sequence linked to the F1 coding region of the truncated RSV F gene. This chimeric gene was subsequently subcloned into the baculovirus expression vector (detailed in Example 5).

Example 5:

This Example illustrates the construction of the modified pAc 610 baculovirus expression vector containing the native polyhedrin promoter and the chimeric F_{PIV-3}-F_{RSV} gene consisting of the PIV-3 F gene lacking both the 5′-untranslated and transmembrane coding sequences linked to the truncated RSV F1 gene.

The pAc 610 baculovirus expression vector was modified to contain the native polyhedrin promoter in the following manner. Vector pAc 610 was cut with EcoRV and BamHI. The 9.4 Kb baculovirus expression vector lacking the EcoRV-BamHI DNA sequence was isolated from a low melting point agarose gel and treated with intestinal alkaline phosphatase. In a 3-way ligation, an EcoRV-EcoRI oligonucleotide cassette (Fig. 8) containing the nucleotides required to restore the native polyhedrin promoter was ligated with the 1.6 Kb EcoRI-BamHI truncated RSV F gene fragment isolated from construct ES13A and the EcoRV-BamHI restricted pAc 610 phosphatased vector to generate plasmid ES47A. To prepare the pAc 610 based expression vector containing the chimeric F_{PIV-3}-F_{RSV} gene, plasmid ES47A was first cut with EcoRI and BamHI to remove the 1.6 Kb truncated RSV F gene insert. The 2.5 Kb F_{PIV-3}-F_{RSV} chimeric gene was retrieved by cutting plasmid ES60A with EcoRI and BamHI. The 2.5 Kb EcoRI-BamHI chimeric gene was ligated to the 7.7 Kb ES47A vector restricted with EcoRI-BamHI to generate plasmid pAc DR7-8.

Example 6:

This Example outlines the preparation of plaque purified recombinant baculoviruses containing the chimeric F_{PIV-3}-F_{RSV} gene.

Spodoptera frugiperda (Sf9) cells were co-transfected with 10 μg wild-type AcMNPV DNA and 2.5 μg of F_{PIV-3}-F_{RSV} plasmid DNA (construct DR7-8). Putative recombinant baculoviruses (purified once by serial dilution) containing the F_{PIV-3}-F_{RSV} chimeric gene were identified by dot-blot hybridization. Lysates of insect cells infected with the putative recombinant baculoviruses were probed with the ^{32}P-labelled F_{PIV-3}-F_{RSV} chimeric gene insert. Recombinant baculoviruses were plaque-purified twice before being used for expression studies. All procedures were carried out

according to the protocols outlined by Summers and Smith in "A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures".

Example 7:

This Example illustrates the presence of the chimeric F_{PIV-3} - F_{RSV} protein in supernatants and cell lysates of infected Sf9 cells.

Insect cells were infected with the plaque purified recombinant baculoviruses at a MOI of 8. Concentrated supernatants from cells infected with the recombinant viruses were positive in a PIV-3 F specific ELISA. In addition, when lysates from ^{35}S methionine labelled infected cells were subjected to SDS-polyacrylamide gel electrophoresis and gels were analyzed by autoradiography, a strong band with expected MW of 90 kDa was present in lysates of cells infected with the recombinant viruses but was absent in the lysates from wild type infected cells. The presence of the chimeric F_{PIV-3} -F_{RSV} protein in the lysates of cells infected with the recombinant baculoviruses was further confirmed by Western blot analysis using anti-PIV-3 F and anti-RSV F monospecific antisera and/or monoclonal antibodies. Lysates from cells infected with the recombinant baculoviruses reacted with both anti-PIV-3 and anti-RSV antisera in immunoblots. As shown in the immunoblot of Fig. 9, lysates from cells infected with either the RSV F or F_{PIV-3}-F_{RSV} recombinant baculoviruses reacted positively with the anti-F RSV Mab. As expected, lysates from cells infected with wild type virus did not react with this Mab. In addition, only lysates from cells infected with the chimeric F_{PIV-3}-F_{RSV} recombinant viruses reacted with the anti-PIV-3 F_1 antiserum.

Example 8:

This Example illustrates the construction of a baculovirus expression vector containing the chimeric F_{RSV}-HN_{PIV-3} gene consisting of the truncated RSV F and

PIV-3 HN genes linked in tandem. In this baculovi expression vector, designated pD2, the polyhedrin A start codon was converted to ATT and the sequence CC was present downstream of the polyhedrin gene at positions $^{+}4$, 5, 6. Insertion of a structural gene several base pairs downstream from the ATT codon is known to enhance translation.

To engineer the F_{RSV}-HN_{PIV-3} gene, the RSV F gene lacking the transmembrane coding region was retrieved from plasmid pRSVF by cutting the polylinker with EcoRI and the gene with BspHI. The PIV-3 HN gene devoid of the hydrophobic anchor domain was retrieved from plasmid pPIVHN by cutting the gene with BspHI and the polylinker with BamHI. The 1.6 Kb EcoRI-BspHI RSV F gene fragment and the 1.7 Kb BspHI-BamHI PIV-3 HN gene fragments were isolated from low melting point agarose gels. For cloning purposes, the two BspHI sites in the Bluescript-based mammalian cell expression vector containing the human methallothionein promoter and poly A and IVS sequences from the SV40 genome were mutated. Mutations were introduced in the BspHI sites of the vector by cutting the expression vector with BspHI, treating both the 1.1 Kb BspHI restricted vector and the 1.1 Kb fragment released by the BspHI cut with Klenow polymerase and ligating the blunt-ended 1.1 Kb fragment to the blunt-ended Bluescript-based expression vector to generate plasmid pM. Since insertion of the 1.1 Kb blunt-end fragment in the mammalian cell expression vector in the improper orientation would alter the amp^{r} gene of the Bluescript-based expression vector, only colonies of HB101 cells transformed with the pM plasmid DNA with the 1.1 Kb blunt-ended fragment in the proper orientation could survive in the presence of ampicillin. Plasmid DNA was purified from ampicillin-resistant colonies of HB101 cells transformed with plasmid pM by equibrium centrifugation in cesium chloride-ethidium

bromide gradients. The 1.6 Kb EcoRI-BspHI RSV F and 1.7 Kb BspHI-BamHI PIV-3 HN gene fragments were ligated via the BspHI site and cloned into the EcoRI-BamHI sites of vector pM to generate plasmid pM RF-HN. To restore specific coding sequences of the RSV F and PIV-3 HN genes removed by the BspHI cut, a BspHI-BspHI oligonucleotide cassette (Fig. 10) containing the pertinent RSV F and PIV-3 HN gene coding sequences was ligated via the BspHI site to the BspHI-restricted plasmid pM RF-HN to produce plasmid pM' RF-HN. Clones containing the BspHI-BspHI oligonucleotide cassette in the proper orientation were identified by sequence analysis of the oligonucleotide linker and its flanking regions. To clone the chimeric F_{RSV}-HN_{PIV-3} gene into the baculovirus expression vector (pD2) in which the ATG of the polyhedrin start codon was converted to ATT, the F_{RSV}-HN_{PIV-3} truncated gene was first retrieved from plasmid pM' RF-HN by cutting the plasmid with EcoRI. The 3.3 Kb F_{RSV}-HN_{PIV-3} gene was then cloned into the EcoRI site of the baculovirus expression vector plasmid pD2 to generate plasmid pD2 RF-HN. Proper orientation of the 3.3 Kb EcoRI F_{RSV}-HN_{PIV-3} chimeric gene insert in plasmid pD2 RF-HN was confirmed by sequence analysis.

Example 9:

This Example outlines the preparation of plaque-purified recombinant baculoviruses containing the chimeric F_{RSV}-HN_{PIV-3} gene.

Spodoptera frugiperda (Sf9) cells were co-transfected with 1 μg wild-type AcNPV DNA and 2 μg of F_{RSV}-HN_{PIV-3} plasmid DNA (construct pD1RF-HN). Putative recombinant baculoviruses (purified once by serial dilution) containing the F_{RSV}-HN_{PIV-3} chimeric gene were identified by dot-blot hybridization. Lysates of insect cells infected with the putative recombinant baculoviruses were probed with the ^{32}P-labelled RSV F or PIV-3 HN gene oligonucleotide probes. Recombinant

baculoviruses were plaque-purified three times before being used for expression studies. All procedures were carried out according to the protocols outlined by Summers and Smith in "A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures".

Example 10:

This Example illustrates the presence of the chimeric F_{RSV}-HN_{PIV-3} protein in supernatants of infected Sf9 cells.

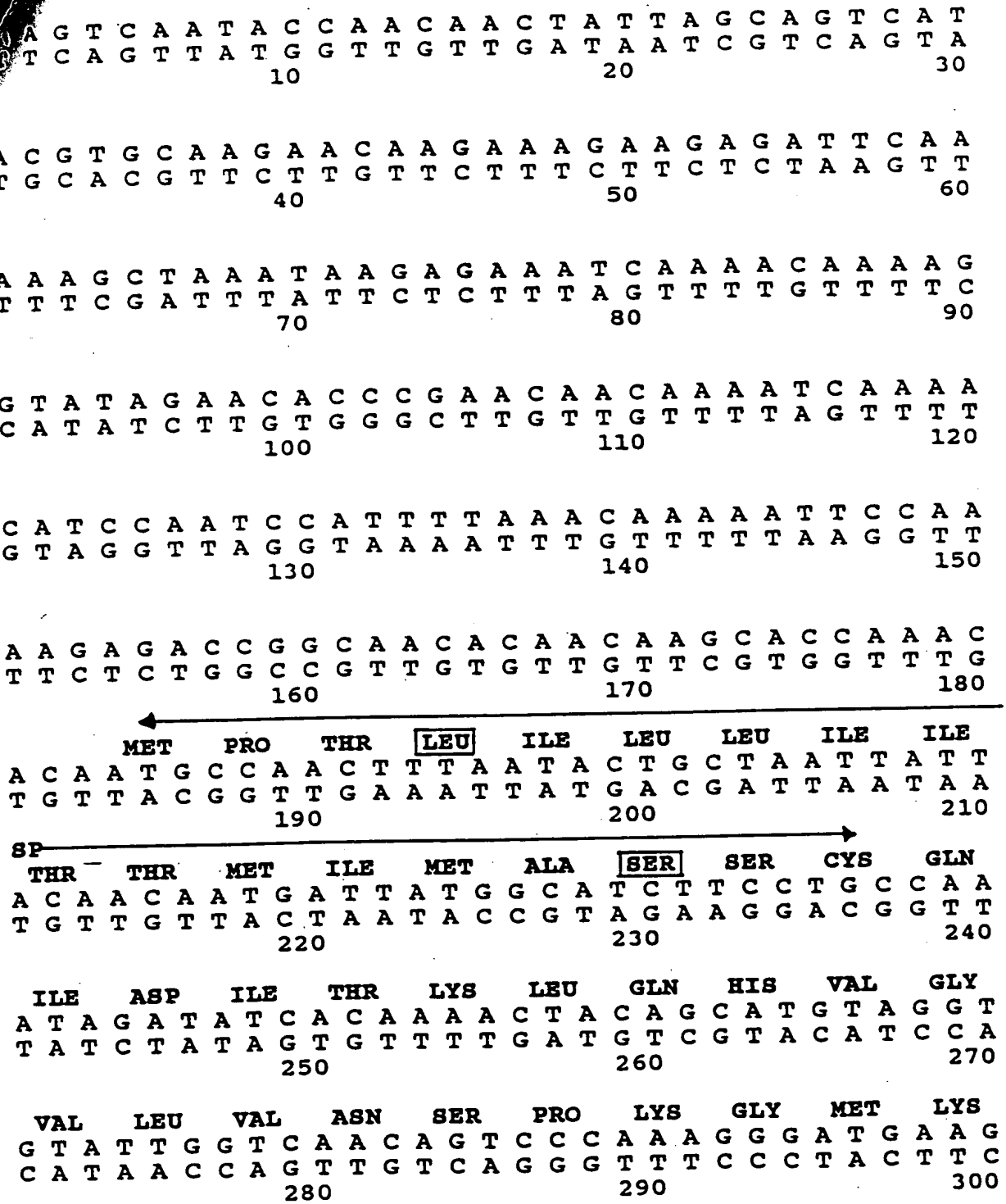
Insect cells, maintained in serum free medium EX401, were infected with the plaque purified recombinant baculoviruses at a MOI of 5 to 10 PFU/cell. Supernatants from cells infected with the recombinant baculoviruses tested positive for expressed protein in both the RSV-F and PIV-3 HN specific ELISAs. In addition, supernatants from infected cells reacted positively with an anti-F RSV monoclonal antibody in immunoblots. A distinct band of approximately 100 kDa was present in the immunoblots. These results confirm the secretion of the chimeric F_{RSV}-HN_{PIV-3} protein into the supernatant of Sf9 cells infected with the recombinant baculoviruses.

It will be apparent from the foregoing disclosure, as illustrated by the Examples, that the inventors have disclosed, in this application, the novel idea of determining the genes in two or more viruses, that are responsible for given antigenic and protective proteins, and joining these together such that, when expressed in a cell system, the resulting product is a chimeric protein that contains the antigenic proteins and which can be used as a vaccine to protect against disease.

The invention has specified proteins and genes from parainfluenza virus and respiratory syncytial virus that are protective when used as immunisation agents, but the invention is not limited to these proteins and the organisms that they have come from. The invention may

be applied to any protein that can be shown to be protective and that can be isolated from any organism, whether bacterial or viral. Modifications are possible within the scope of this invention.

FIGURE 1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F2-F1 Cleavage site
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                  670                  680                  690

 VAL  ALA  ILE  LYS  SER  VAL  GLN  ASP  TYR  VAL
G T A G C A A T T A A A T C A G T C C A A G A T T A T G T C
C A T C G T T A A T T T A G T C A G G T T C T A A T A C A G
                  700                  710                  720

 ASN  [ASN]  GLU  MET  VAL  PRO  SER  ILE  ALA  ARG
A A C A A C G A A A T G G T G C C A T C G A T T G C T A G A
T T G T T G C T T T A C C A C G G T A G C T A A C G A T C T
                  730                  740                  750

 LEU  GLY  CYS  GLU  ALA  ALA  GLY  LEU  GLN  LEU
C T A G G T T G T G A A G C A G C A G G A C T T C A A T T A
G A T C C A A C A C T T C G T C G T C C T G A A G T T A A T
                  760                  770                  780

 GLY  ILE  ALA  LEU  THR  GLN  HIS  TYR  SER  GLU
G G A A T T G C A T T A A C A C A G C A T T A C T C A G A A
C C T T A A C G T A A T T G T G T C G T A A T G A G T C T T
                  790                  800                  810

 LEU  THR  ASN  ILE  PHE  GLY  ASP  ASN  ILE  GLY
T T A A C A A A C A T A T T T G G T G A T A A C A T A G G A
A A T T G T T T G T A T A A A C C A C T A T T G T A T C C T
                  820                  830                  840

 SER  LEU  GLN  GLU  LYS  GLY  ILE  LYS  LEU  GLN
T C G T T A C A A G A A A A A G G A A T A A A A T T A C A A
A G C A A T G T T C T T T T T C C T T A T T T T A A T G T T
                  850                  860                  870

 GLY  ILE  ALA  SER  LEU  TYR  ARG  THR  ASN  ILE
G G T A T A G C A T C A T T A T A C C G C A C A A A T A T C
C C A T A T C G T A G T A A T A T G G C G T G T T T A T A G
                  880                  890                  900
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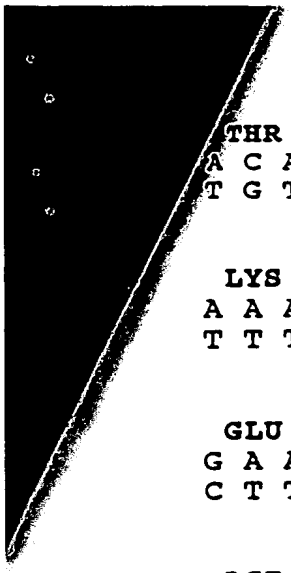

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THR   GLU   ILE   PHE   THR   THR   SER   THR   VAL   ASP
A C A G A A A T A T T C A C A A C A T C A A C A G T T G A T
T G T C T T T A T A A G T G T T G T A G T T G T C A A C T A
                  910                 920                 930

 LYS   TYR   ASP   ILE   TYR   ASP   LEU   LEU   PHE   THR
A A A T A T G A T A T C T A T G A T C T A T T A T T T A C A
T T T A T A C T A T A G A T A C T A G A T A A T A A A T G T
                  940                 950                 960

 GLU   SER   ILE   LYS   VAL   ARG   VAL   ILE   ASP   VAL
G A A T C A A T A A A G G T G A G A G T T A T A G A T G T T
C T T A G T T A T T T C C A C T C T C A A T A T C T A C A A
                  970                 980                 990

 ASP   LEU   ASN   ASP   TYR   SER   ILE   THR   LEU   GLN
G A T T T G A A T G A T T A C T C A A T C A C C C T C C A A
C T A A A C T T A C T A A T G A G T T A G T G G G A G G T T
                 1000                1010                1020

 VAL   ARG   LEU   PRO   LEU   LEU   THR   ARG   LEU   LEU
G T C A G A C T C C C T T T A T T A A C T A G G C T G C T G
C A G T C T G A G G G A A A T A A T T G A T C C G A C G A C
                 1030                1040                1050

 ASN   THR   GLN   ILE   TYR  [LYS]  VAL   ASP   SER   ILE
A A C A C T C A G A T C T A C A A A G T A G A T T C C A T A
T T G T G A G T C T A G A T G T T T C A T C T A A G G T A T
                 1060                1070                1080

 SER   TYR   ASN   ILE   GLN   ASN   ARG   GLU   TRP   TYR
T C A T A T A A T A T C C A A A A C A G A G A A T G G T A T
A G T A T A T T A T A G G T T T T G T C T C T T A C C A T A
                 1090                1100                1110

 ILE   PRO   LEU   PRO   SER   HIS   ILE   MET   THR   LYS
A T C C C T C T T C C C A G C C A T A T C A T G A C G A A A
T A G G G A G A A G G G T C G G T A T A G T A C T G C T T T
                 1120                1130                1140

 GLY   ALA   PHE   LEU   GLY   GLY   ALA   ASP   VAL   LYS
G G G G C A T T T C T A G G T G G A G C A G A T G T C A A G
C C C C G T A A A G A T C C A C C T C G T C T A C A G T T C
                 1150                1160                1170

 GLU   CYS   ILE   GLU   ALA   PHE   SER   SER   TYR   ILE
G A A T G T A T A G A A G C A T T C A G C A G T T A T A T A
C T T A C A T A T C T T C G T A A G T C G T C A A T A T A T
                 1180                1190                1200
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YB   PRO   SER   ASP   PRO   GLY   PHE   VAL   LEU   ASN
G C C C T T C T G A T C C A G G A T T T G T A C T A A A C
C G G G A A G A C T A G G T C C T A A A C A T G A T T T G
                1210                1220                1230

 HIS   GLU   MET   GLU   SER   CYS   LEU   SER   GLY   ASN
C A T G A A A T G G A G A G C T G C T T A T C A G G A A A C
G T A C T T T A C C T C T C G A C G A A T A G T C C T T T G
                1240                1250                1260

 ILE   SER   GLN   CYS   PRO   ARG   THR  [THR]  VAL  [THR]
A T A T C C C A A T G T C C A A G A A C C A C G G T C A C A
T A T A G G G T T A C A G G T T C T T G G T G C C A G T G T
                1270                1280                1290

 SER   ASP   ILE   VAL   PRO   ARG   TYR   ALA   PHE   VAL
T C A G A C A T T G T T C C A A G A T A T G C A T T T G T C
A G T C T G T A A C A A G G T T C T A T A C G T A A A C A G
                1300                1310                1320

 ASN   GLY   GLY   VAL   VAL   ALA   ASN   CYS   ILE   THR
A A T G G A G G A G T G G T T G C A A A C T G T A T A A C A
T T A C C T C C T C A C C A A C G T T T G A C A T A T T G T
                1330                1340                1350

 THR   THR   CYS   THR   CYS   ASN   GLY   ILE  [ASP]  ASN
A C C A C C T G T A C A T G C A A C G G A A T C G A C A A T
T G G T G G A C A T G T A C G T T G C C T T A G C T G T T A
                1360                1370                1380

 ARG   ILE   ASN   GLN   PRO   PRO   ASP   GLN   GLY   VAL
A G A A T C A A T C A A C C A C C T G A T C A A G G A G T A
T C T T A G T T A G T T G G T G G A C T A G T T C C T C A T
                1390                1400                1410

 LYS   ILE   ILE   THR   HIS   LYS   GLU   CYS   ASN   THR
A A A A T T A T A A C A C A T A A A G A A T G T A A T A C A
T T T T A A T A T T G T G T A T T T C T T A C A T T A T G T
                1420                1430                1440

 ILE   GLY   ILE   ASN   GLY   MET   LEU   PHE   ASN   THR
A T A G G T A T C A A C G G A A T G C T G T T C A A T A C A
T A T C C A T A G T T G C C T T A C G A C A A G T T A T G T
                1450                1460                1470

 ASN   LYS   GLU   GLY   THR   LEU   ALA   PHE   TYR   THR
A A T A A A G A A G G A A C T C T T G C A T T C T A C A C A
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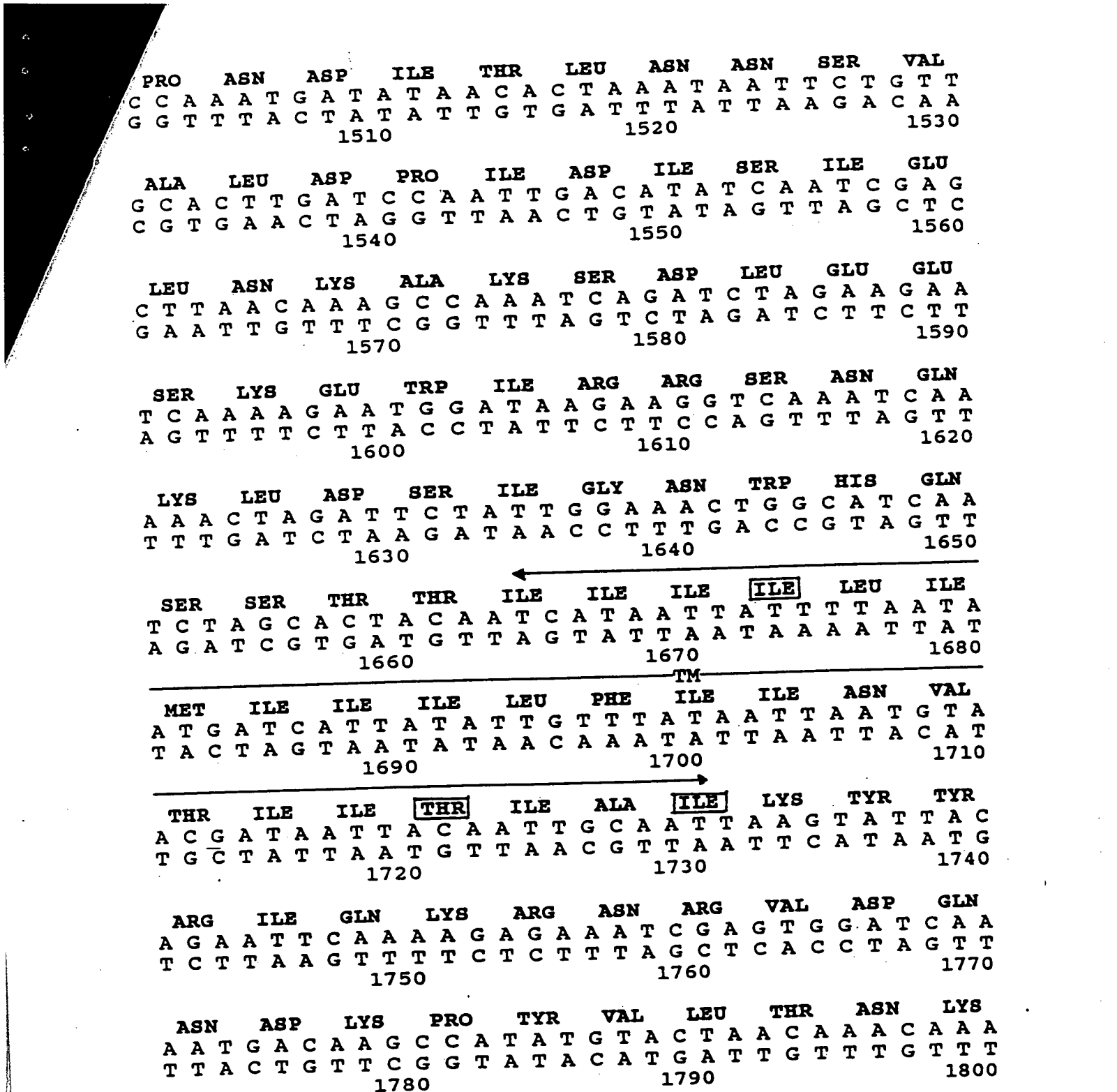
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C C A A A T G A T A T A A C A C T A A A T A A T T C T G T T
G G T T T A C T A T A T T G T G A T T T A T T A A G A C A A
1510 1520 1530
ALA LEU ASP PRO ILE ASP ILE SER ILE GLU
G C A C T T G A T C C A A T T G A C A T A T C A A T C G A G
C G T G A A C T A G G T T A A C T G T A T A G T T A G C T C
1540 1550 1560
LEU ASN LYS ALA LYS SER ASP LEU GLU GLU
C T T A A C A A A G C C A A A T C A G A T C T A G A A G A A
G A A T T G T T T C G G T T T A G T C T A G A T C T T C T T
1570 1580 1590
SER LYS GLU TRP ILE ARG ARG SER ASN GLN
T C A A A A G A A T G G A T A A G A A G G T C A A A T C A A
A G T T T T C T T A C C T A T T C T T C C A G T T T A G T T
1600 1610 1620
LYS LEU ASP SER ILE GLY ASN TRP HIS GLN
A A A C T A G A T T C T A T T G G A A A C T G G C A T C A A
T T T G A T C T A A G A T A A C C T T T G A C C G T A G T T
1630 1640 1650
SER SER THR THR ILE ILE ILE ILE LEU ILE
T C T A G C A C T A C A A T C A T A A T T A T T T T A A T A
A G A T C G T G A T G T T A G T A T T A A T A A A A A T T A T
1660 1670 1680
TM
MET ILE ILE ILE LEU PHE ILE ILE ASN VAL
A T G A T C A T T A T A T T G T T T A T A A T T A A T G T A
T A C T A G T A A T A T A A C A A A T A T T A A T T A C A T
1690 1700 1710
THR ILE ILE THR ILE ALA ILE LYS TYR TYR
A C G A T A A T T A C A A T T G C A A T T A A G T A T T A C
T G C T A T T A A T G T T A A C G T T A A T T C A T A A T G
1720 1730 1740
ARG ILE GLN LYS ARG ASN ARG VAL ASP GLN
A G A A T T C A A A A G A G A A A T C G A G T G G A T C A A
T C T T A A G T T T T C T C T T T A G C T C A C C T A G T T
1750 1760 1770
ASN ASP LYS PRO TYR VAL LEU THR ASN LYS
A A T G A C A A G C C A T A T G T A C T A A C A A A C A A A
T T A C T G T T C G G T A T A C A T G A T T G T T T G T T T
1780 1790 1800

```
****
T G A C A T A T C T A T A G A T C A T T A G A T A T T A A A
A C T G T A T A G A T A T C T A G T A A T C T A T A A T T T
                1810                1820                1830

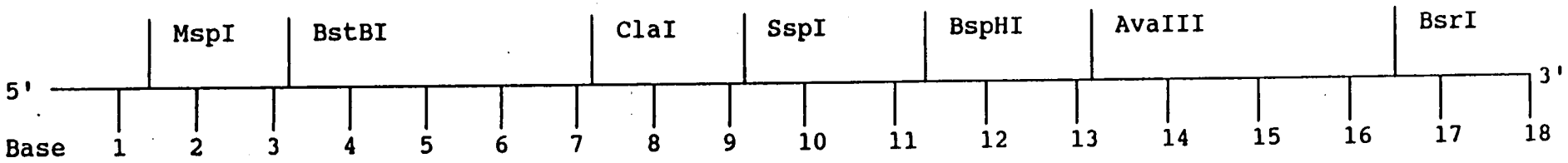
                                HN gene ----------

A T T A T A A A A A A C T T A G G A G T A A A G T T A C G C
T A A T A T T T T T G A A T C C T C A T T T C A A T G C G
                1840                1850                1860

A A T C C A A C T C T A C T C A T A T A A T T G A C G G G
T T A G G T T G A G A T G A G T A T A T T A A C T G C C C
                1870                1880
```

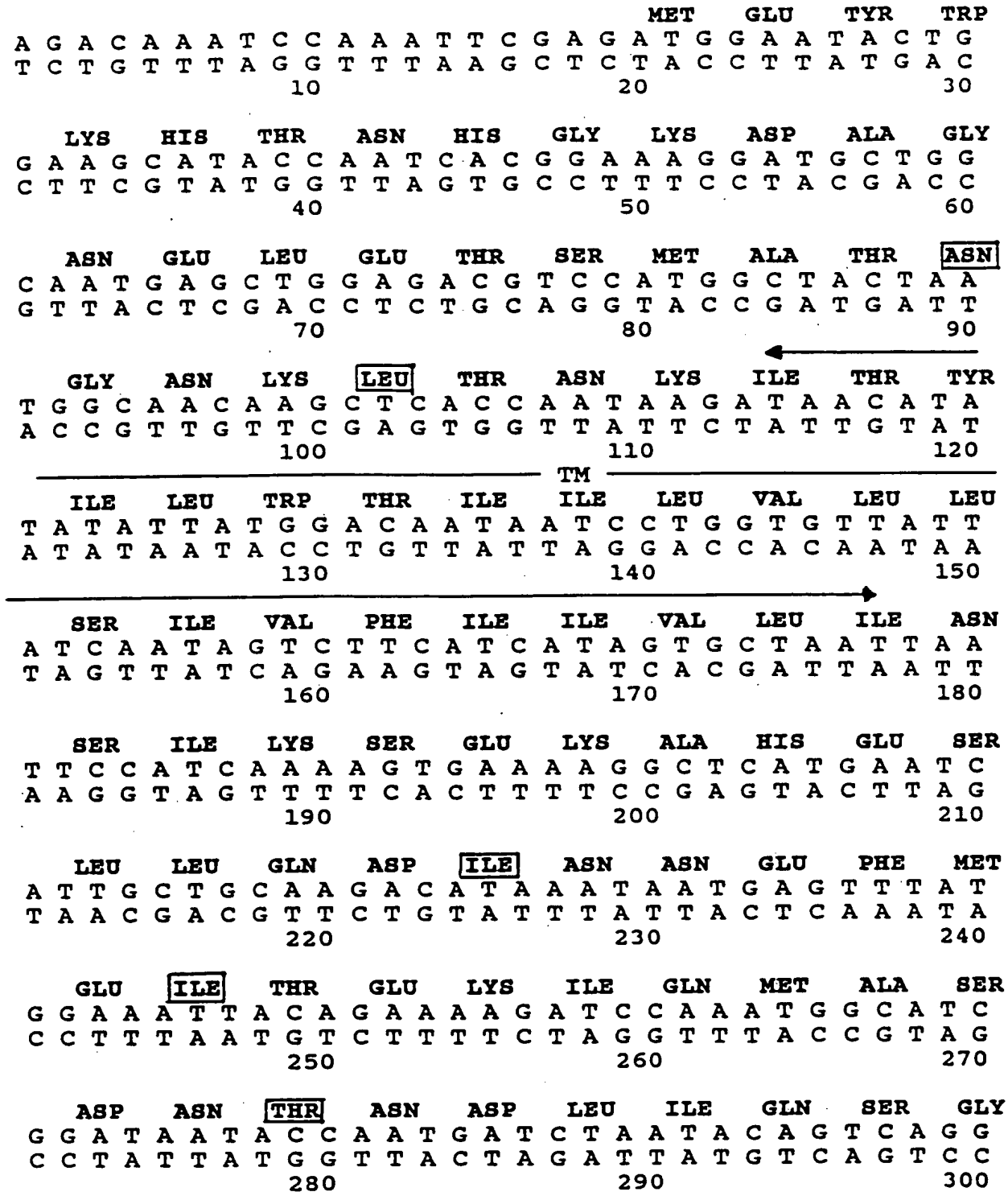
Figure 1: Nucleotide and amino acid sequences of the PIV-3 F gene. The cDNA sequence is shown in the plus (mRNA) strand sense in the 5' to 3' direction. The signal peptide (SP) and the transmembrane (TM) anchor domain are underlined. The predicted F2-F1 cleavage site is indicated by the arrow (↓). Amino acids differing from the published coding sequence of the PIV-3 F gene are boxed.

FIGURE 2: RESTRICTION MAP OF THE PIV-3 F GENE



1.0 cm = 100 bases

FIGURE 3.


MET GLU TYR TRP
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T C T G T T T A G G T T T A A G C T C T A C C T T A T G A C
10 20 30
LYS HIS THR ASN HIS GLY LYS ASP ALA GLY
G A A G C A T A C C A A T C A C G G A A A G G A T G C T G G
C T T C G T A T G G T T A G T G C C T T T C C T A C G A C C
40 50 60
ASN GLU LEU GLU THR SER MET ALA THR ASN
C A A T G A G C T G G A G A C G T C C A T G G C T A C T A A
G T T A C T C G A C C T C T G C A G G T A C C G A T G A T T
70 80 90
GLY ASN LYS LEU THR ASN LYS ILE THR TYR
T G G C A A C A A G C T C A C C A A T A A G A T A A C A T A
A C C G T T G T T C G A G T G G T T A T T C T A T T G T A T
100 110 120
TM
ILE LEU TRP THR ILE ILE LEU VAL LEU LEU
T A T A T T A T G G A C A A T A A T C C T G G T G T T A T T
A T A T A A T A C C T G T T A T T A G G A C C A C A A T A A
130 140 150
SER ILE VAL PHE ILE ILE VAL LEU ILE ASN
A T C A A T A G T C T T C A T C A T A G T G C T A A T T A A
T A G T T A T C A G A A G T A G T A T C A C G A T T A A T T
160 170 180
SER ILE LYS SER GLU LYS ALA HIS GLU SER
T T C C A T C A A A A G T G A A A A G G C T C A T G A A T C
A A G G T A G T T T T C A C T T T T C C G A G T A C T T A G
190 200 210
LEU LEU GLN ASP ILE ASN ASN GLU PHE MET
A T T G C T G C A A G A C A T A A A T A A T G A G T T T A T
T A A C G A C G T T C T G T A T T T A T T A C T C A A A T A
220 230 240
GLU ILE THR GLU LYS ILE GLN MET ALA SER
G G A A A T T A C A G A A A A G A T C C A A A T G G C A T C
C C T T T A A T G T C T T T T C T A G G T T T A C C G T A G
250 260 270
ASP ASN THR ASN ASP LEU ILE GLN SER GLY
G G A T A A T A C C A A T G A T C T A A T A C A G T C A G G
C C T A T T A T G G T T A C T A G A T T A T G T C A G T C C
280 290 300

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      VAL   ASN   THR   ARG   LEU   LEU   THR   ILE   GLN   SER
AGTGAATACAAGGCTTCTTACAATTCAGAG
TCACTTATGTTCCGAAGAATGTTAAGTCTC
          310               320               330

      HIS   VAL   GLN   ASN   TYR   ILE   PRO   ILE   SER   LEU
TCATGTCCAGAATTATATACCAATATCACT
AGTACAGGTCTTAATATATGGTTATAGTGA
          340               350               360

      THR   GLN   GLN   MET   SER   ASP   LEU   ARG   LYS   PHE
GACACAACAGATGTCAGATCTTAGGAAATT
CTGTGTTGTCTACAGTCTAGAATCCTTTAA
          370               380               390

      ILE   SER   GLU   ILE   THR   ILE   ARG   ASN   ASP   ASN
CATTAGTGAAATTACAATTAGAAATGATAA
GTAATCACTTTAATGTTAATCTTTACTATT
          400               410               420

      [GLN]  GLU   VAL  [LEU]  PRO   GLN   ARG   ILE   THR   HIS
TCAAGAAGTGCTGCCACAAAGAATAACACA
AGTTCTTCACGACGGTGTTTCTTATTGTGT
          430               440               450

      ASP  [VAL]  GLY   ILE   LYS   PRO   LEU   ASN   PRO   ASP
TGATGTGGGTATAAAACCTTTAAATCCAGA
ACTACACCCATATTTTGGAAATTTAGGTCT
          460               470               480

      ASP   PHE   TRP   ARG   CYS   THR   SER   GLY   LEU   PRO
TGATTTTTGGAGATGCACGTCTGGTCTTCC
ACTAAAAACCTCTACGTGCAGACCAGAAGG
          490               500               510

      SER   LEU   MET   LYS   THR   PRO   LYS   ILE   ARG   LEU
ATCTTTAATGAAAACTCCAAAAATAAGGTT
TAGAAATTACTTTTGAGGTTTTTATTCCAA
          520               530               540

      MET   PRO   GLY   PRO   GLY   LEU   LEU   ALA   MET   PRO
AATGCCAGGGCCGGGATTATTAGCTATGCC
TTACGGTCCCGGCCCTAATAATCGATACGG
          550               560               570

      THR   THR   VAL   ASP   GLY   CYS  [ILE]  ARG   THR   PRO
AACGACTGTTGATGGCTGTATCAGAACTCC
TTGCTGACAACTACCGACATAGTCTTGAGG
          580               590               600
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   SER   LEU   VAL   ILE   ASN   ASP   LEU   ILE   TYR   ALA
G T C C T T A G T T A T A A A T G A T C T G A T T T A T G C
C A G G A A T C A A T A T T T A C T A G A C T A A A T A C G
                  610                   620                   630

   TYR   THR   SER   ASN   LEU   ILE   THR   ARG   GLY   CYS
T T A T A C C T C A A A T C T A A T T A C T C G A G G T T G
A A T A T G G A G T T T A G A T T A A T G A G C T C C A A C
                  640                   650                   660

   GLN   ASP   ILE   GLY   LYS   SER   TYR   GLN   VAL   LEU
T C A G G A T A T A G G A A A A T C A T A T C A A G T C T T
A G T C C T A T A T C C T T T T A G T A T A G T T C A G A A
                  670                   680                   690

   GLN   ILE   GLY   ILE   ILE   THR   VAL   ASN   SER   ASP
A C A G A T A G G G A T A A T A A C T G T A A A C T C A G A
T G T C T A T C C C T A T T A T T G A C A T T T G A G T C T
                  700                   710                   720

   LEU   VAL   PRO   ASP   LEU   ASN   PRO   ARG   ILE   SER
C T T G G T A C C T G A C T T A A A T C C C A G G A T C T C
G A A C C A T G G A C T G A A T T T A G G G T C C T A G A G
                  730                   740                   750

   HIS   THR   PHE   ASN   ILE   ASN   ASP   ASN   ARG   LYS
T C A T A C T T T T A A C A T A A A T G A C A A T A G G A A
A G T A T G A A A A T T G T A T T T A C T G T T A T C C T T
                  760                   770                   780

   SER   CYS   SER   LEU   ALA   LEU   LEU   ASN   THR   ASP
G T C A T G T T C T C C T A G C A C T C C T A A A T A C A G A
C A G T A C A A G A G A T C G T G A G G A T T T A T G T C T
                  790                   800                   810

   VAL   TYR   GLN   LEU   CYS   SER   THR   PRO   LYS   VAL
T G T A T A T C A A C T G T G T T C A A C T C C C A A A G T
A C A T A T A G T T G A C A C A A G T T G A G G G T T T C A
                  820                   830                   840

   ASP   GLU   ARG   SER   ASP   TYR   ALA   SER   SER   GLY
T G A T G A A A G A T C A G A T T A T G C A T C A T C A G G
A C T A C T T T C T A G T C T A A T A C G T A G T A G T C C
                  850                   860                   870

   ILE   GLU   ASP   ILE   VAL   LEU   ASP   ILE   VAL   ASN
C A T A G A A G A T A T T G T A C T T G A T A T T G T C A A
G T A T C T T C T A T A A C A T G A A C T A T A A C A G T T
                  880                   890                   900
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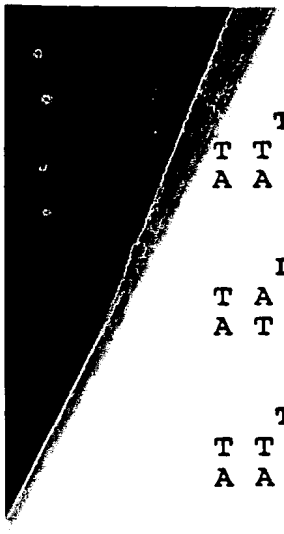

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   TYR   ASP   GLY   SER   ILE   SER   THR   THR   ARG   PHE
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A A T A C T A C C G A G T T A G A G T T G T T G T T C T A A
                  910                 920                 930

   LYS   ASN   ASN   ASN   ILE   SER   PHE   ASP   GLN   PRO
T A A G A A T A A T A A C A T A A G C T T T G A T C A A C C
A T T C T T A T T A T T G T A T T C G A A A C T A G T T G G
                  940                 950                 960

   TYR   ALA   ALA   LEU   TYR   PRO   SER   VAL   GLY   PRO
T T A T G C T G C A C T A T A C C C A T C T G T T G G A C C
A A T A C G A C G T G A T A T G G G T A G A C A A C C T G G
                  970                 980                 990

   GLY   ILE   TYR   TYR   LYS   GLY   LYS   ILE   ILE   PHE
A G G G A T A T A C T A C A A A G G C A A A A T A A T A T T
T C C C T A T A T G A T G T T T C C G T T T T A T T A T A A
                 1000                1010                1020

   LEU   GLY   TYR   GLY   GLY   LEU   GLU   HIS   PRO   ILE
T C T C G G G T A T G G A G G T C T T G A A C A T C C A A T
A G A G C C C A T A C C T C C A G A A C T T G T A G G T T A
                 1030                1040                1050

   ASN   GLU   ASN   VAL   ILE   CYS   ASN   THR   THR   GLY
A A A T G A G A A T G T A A T C T G C A A C A C A A C T G G
T T T A C T C T T A C A T T A G A C G T T G T G T T G A C C
                 1060                1070                1080

   CYS   PRO   GLY   LYS   THR   GLN   ARG   ASP   CYS   ASN
G T G T C C C G G G A A A A C A C A G A G A G A C T G C A A
C A C A G G G C C C T T T T G T G T C T C T C T G A C G T T
                 1090                1100                1110

   GLN   ALA   SER   HIS   SER   PRO   TRP   PHE   SER   ASP
T C A G G C A T C T C A T A G T C C A T G G T T T T C A G A
A G T C C G T A G A G T A T C A G G T A C C A A A A G T C T
                 1120                1130                1140

   ARG   ARG   MET   VAL   ASN   SER   ILE   ILE   VAL   VAL
T A G G A G G A T G G T C A A C T C T A T C A T T G T T G T
A T C C T C C T A C C A G T T G A G A T A G T A A C A A C A
                 1150                1160                1170

   ASP   LYS   GLY   LEU   ASN   SER   ILE   PRO   LYS   LEU
T G A C A A A G G C T T A A A C T C A A T T C C A A A A T T
A C T G T T T C C G A A T T T G A G T T A A G G T T T T A A
                 1180                1190                1200
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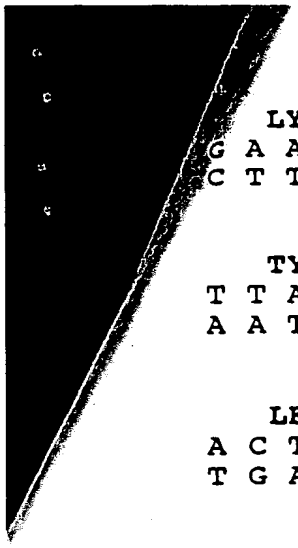

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  LYS   VAL   TRP   THR   ILE   SER   MET   ARG   GLN   ASN
G A A G G T A T G G A C G A T A T C T A T G A G A C A G A A
C T T C C A T A C C T G C T A T A G A T A C T C T G T C T T
                1210                1220                1230

  TYR   TRP   GLY   SER   GLU   GLY   ARG   LEU   LEU   LEU
T T A C T G G G G G T C A G A A G G A A G G T T A C T T C T
A A T G A C C C C C A G T C T T C C T T C C A A T G A A G A
                1240                1250                1260

  LEU   GLY   ASN   LYS   ILE   TYR   ILE   TYR   THR   ARG
A C T A G G T A A C A A G A T C T A T A T A T A T A C A A G
T G A T C C A T T G T T C T A G A T A T A T A T A T G T T C
                1270                1280                1290

  SER   THR   SER   TRP   HIS   SER   LYS   LEU   GLN   LEU
A T C C A C A A G T T G G C A T A G C A A G T T A C A A T T
T A G G T G T T C A A C C G T A T C G T T C A A T G T T A A
                1300                1310                1320

  GLY   ILE   ILE   ASP   ILE   THR   ASP   TYR   SER   ASP
A G G A A T A A T T G A T A T T A C T G A T T A C A G T G A
T C C T T A T T A A C T A T A A T G A C T A A T G T C A C T
                1330                1340                1350

  ILE   ARG   ILE   LYS   TRP   THR   TRP   HIS   ASN   VAL
T A T A A G G A T A A A A T G G A C A T G G C A T A A T G T
A T A T T C C T A T T T T A C C T G T A C C G T A T T A C A
                1360                1370                1380

  LEU   SER   ARG   PRO   GLY   ASN   ASN   GLU   CYS   PRO
G C T A T C A A G A C C A G G A A A C A A T G A A T G T C C
C G A T A G T T C T G G T C C T T T G T T A C T T A C A G G
                1390                1400                1410

  TRP   GLY   HIS   SER   CYS   PRO   ASP   GLY   CYS   ILE
A T G G G G A C A T T C A T G T C C A G A T G G A T G T A T
T A C C C C T G T A A G T A C A G G T C T A C C T A C A T A
                1420                1430                1440

  THR   GLY   VAL   TYR   THR   ASP   ALA   TYR   PRO   LEU
A A C A G G A G T A T A T A C T G A T G C A T A T C C A C T
T T G T C C T C A T A T A T G A C T A C G T A T A G G T G A
                1450                1460                1470

  ASN   PRO   THR   GLY   SER   ILE   VAL   SER   SER   VAL
C A A T C C C A C A G G G A G C A T T G T G T C A T C T G T
G T T A G G G T G T C C C T C G T A A C A C A G T A G A C A
                1480                1490                1500
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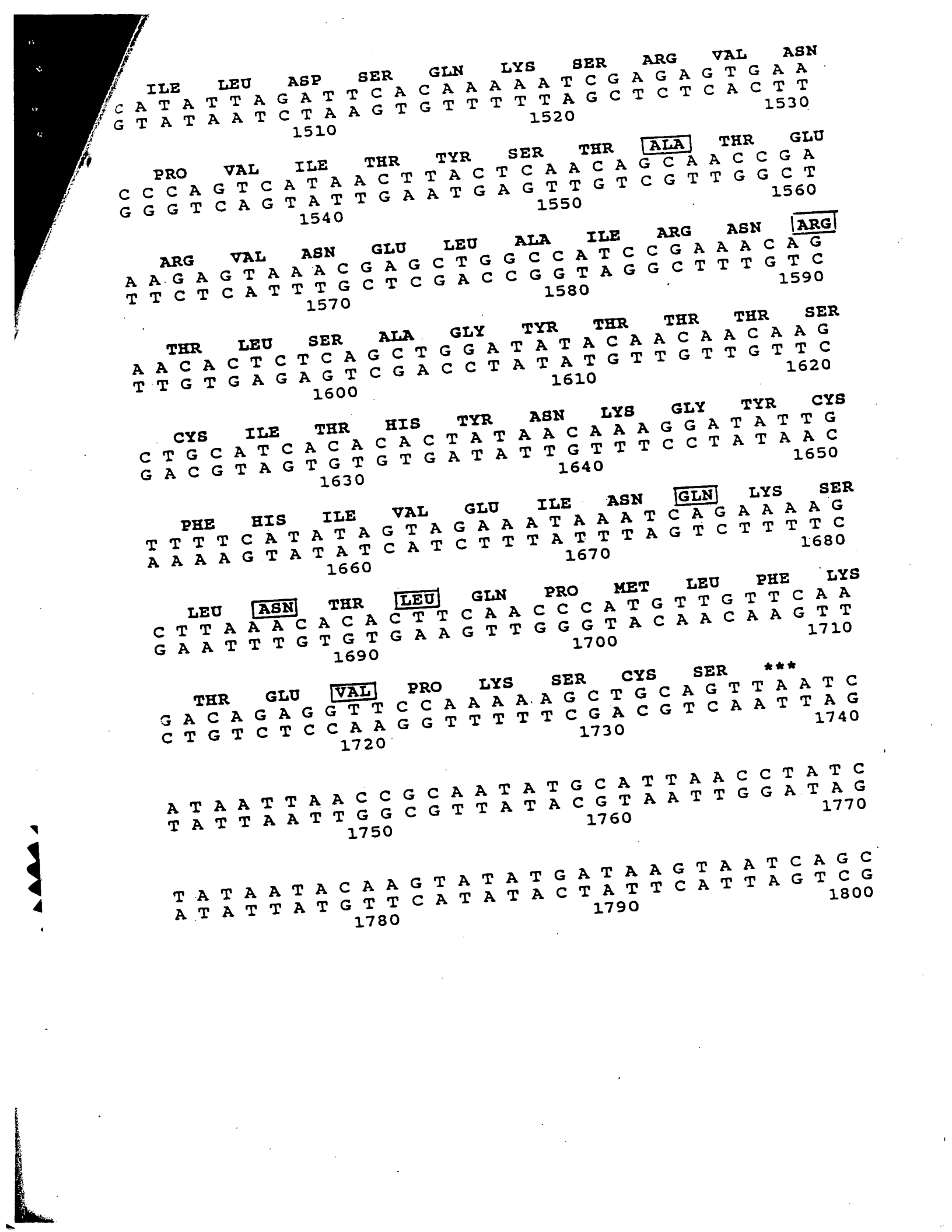
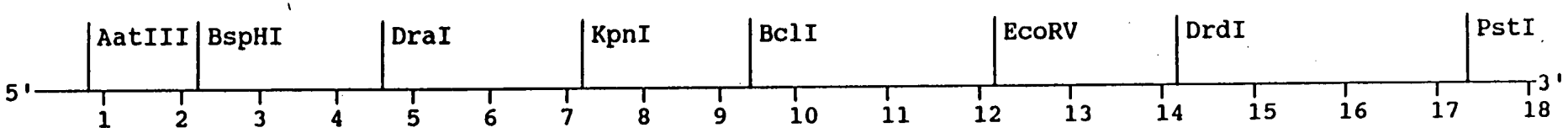

ILE LEU ASP SER GLN LYS SER ARG VAL ASN
CATATTAGATTCACAAAAAATCGAGAGTGAAA
GTATAATCTAAGTGTTTTTAGCTCTCACTT
1510 1520 1530
PRO VAL ILE THR TYR SER THR ALA THR GLU
CCCAGTCATAACTTACTCAACAGCAACCGA
GGGTCAGTATTGAATGAGTTGTCGTTGGCT
1540 1550 1560
ARG VAL ASN GLU LEU ALA ILE ARG ASN ARG
AAGAGTAAACGAGCTGGCCATCCGAAACAG
TTCTCATTTGCTCGACCGGTAGGCTTTGTC
1570 1580 1590
THR LEU SER ALA GLY TYR THR THR THR SER
AACACTCTCAGCTGGATATACAACAACAAG
TTGTGAGAGTCGACCTATATGTTGTTGTTC
1600 1610 1620
CYS ILE THR HIS TYR ASN LYS GLY TYR CYS
CTGCATCACACTATAACAAAGGATATTG
GACGTAGTGTGATATTGTTTCCTATAAC
1630 1640 1650
PHE HIS ILE VAL GLU ILE ASN GLN LYS SER
TTTTCATATAGTAGAAATAAATCAGAAAAAG
AAAAGTATATCATCTTTATTTAGTCTTTTC
1660 1670 1680
LEU ASN THR LEU GLN PRO MET LEU PHE LYS
CTTAAACACACTTCAACCCATGTTGTTCAA
GAATTTGTGTGAAGTTGGGTACAACAAGTT
1690 1700 1710
THR GLU VAL PRO LYS SER CYS SER ***
GACAGAGGTTCCAAAAAGCTGCAGTTAATC
CTGTCTCCAAGGTTTTTCGACGTCAATTAG
1720 1730 1740
ATAATTAACCGCAATATGCATTAACCTATC
TATTAATTGGCGTTATACGTAATTGGATAG
1750 1760 1770
TATAATACAAGTATATGATAAGTAATCAGC
ATATTATGTTCATATACTATTCATTAGTCG
1780 1790 1800

A A T C A G A C A A T A G A C A A A A G G G A A A T A T A A
T T A G T C T G T T A T C T G T T T T C C C T T T A T A T T
1810 1820 1830

A A A
T T T

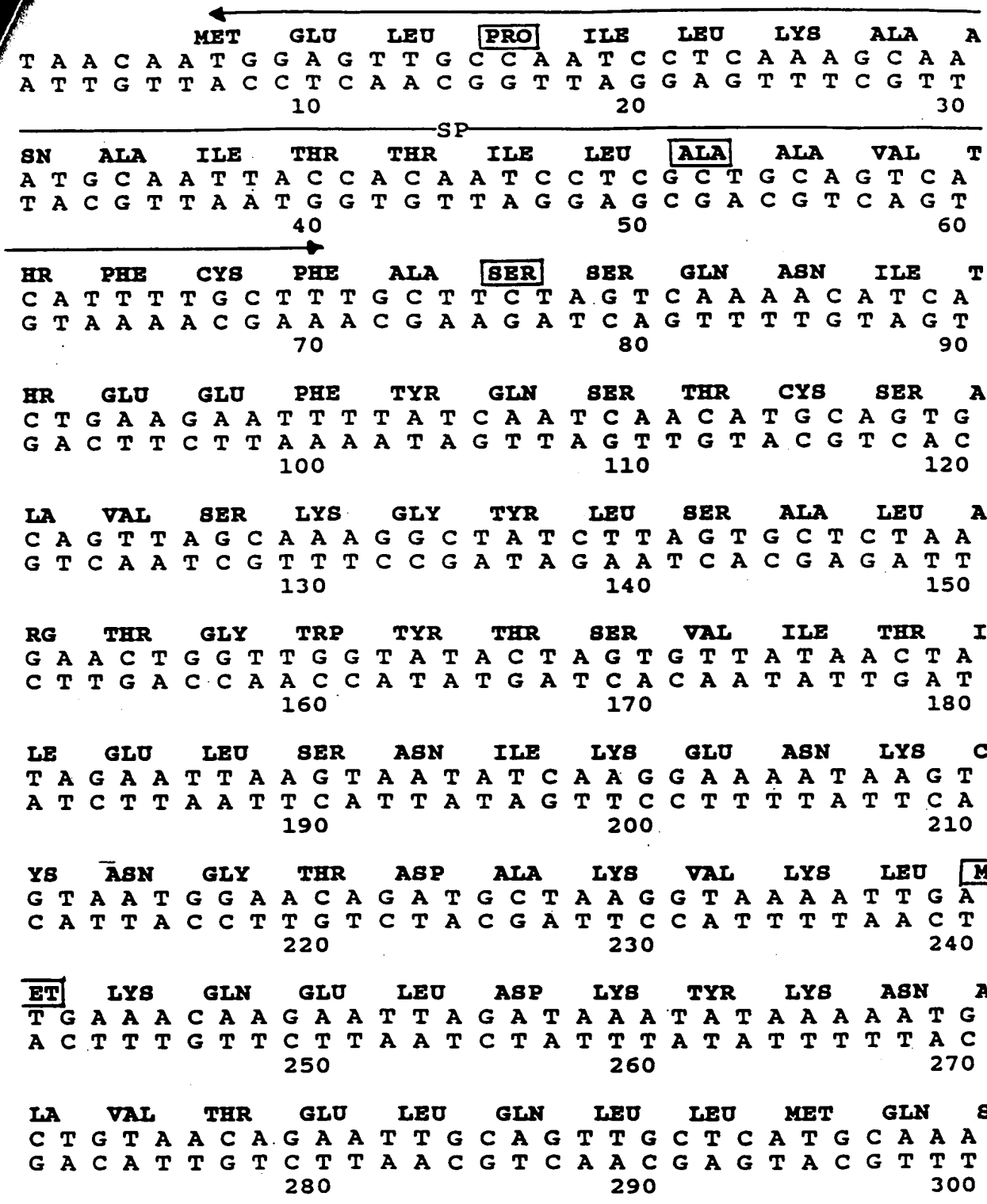
Figure 3: Nucleotide and amino acid sequences of the PIV-3 HN gene. The cDNA sequence is shown in the plus (mRNA) strand sense in the 5' to 3' direction. The transmembrane (TM) anchor domain is underlined. Amino acids differing from the published coding sequence of the PIV-3 HN gene are boxed.

FIGURE 4: RESTRICTION MAP OF THE RSV F GENE



1.0 cm = 100 bases

FIGURE 5.


MET GLU LEU PRO ILE LEU LYS ALA A
T A A C A A T G G A G T T G C C A A T C C T C A A A G C A A
A T T G T T A C C T C A A C G G T T A G G A G T T T C G T T
10 20 30
SP
SN ALA ILE THR THR ILE LEU ALA ALA VAL T
A T G C A A T T A C C A C A A T C C T C G C T G C A G T C A
T A C G T T A A T G G T G T T A G G A G C G A C G T C A G T
40 50 60
HR PHE CYS PHE ALA SER SER GLN ASN ILE T
C A T T T T G C T T T G C T T C T A G T C A A A A A C A T C A
G T A A A A C G A A A C G A A G A T C A G T T T T G T A G T
70 80 90
HR GLU GLU PHE TYR GLN SER THR CYS SER A
C T G A A G A A T T T T A T C A A T C A A C A T G C A G T G
G A C T T C T T A A A A T A G T T A G T T G T A C G T C A C
100 110 120
LA VAL SER LYS GLY TYR LEU SER ALA LEU A
C A G T T A G C A A A G G C T A T C T T A G T G C T C T A A
G T C A A T C G T T T C C G A T A G A A T C A C G A G A T T
130 140 150
RG THR GLY TRP TYR THR SER VAL ILE THR I
G A A C T G G T T G G T A T A C T A G T G T T A T A A C T A
C T T G A C C A A C C A T A T G A T C A C A A T A T T G A T
160 170 180
LE GLU LEU SER ASN ILE LYS GLU ASN LYS C
T A G A A T T A A G T A A T A T C A A G G A A A A T A A G T
A T C T T A A T T C A T T A T A G T T C C T T T T A T T C A
190 200 210
YS ASN GLY THR ASP ALA LYS VAL LYS LEU M
G T A A T G G A A C A G A T G C T A A G G T A A A A T T G A
C A T T A C C T T G T C T A C G A T T C C A T T T T A A C T
220 230 240
ET LYS GLN GLU LEU ASP LYS TYR LYS ASN A
T G A A A C A A G A A T T A G A T A A A T A T A A A A A T G
A C T T T G T T C T T A A T C T A T T T A T A T T T T T A C
250 260 270
LA VAL THR GLU LEU GLN LEU LEU MET GLN S
C T G T A A C A G A A T T G C A G T T G C T C A T G C A A A
G A C A T T G T C T T A A C G T C A A C G A G T A C G T T T
280 290 300

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ER   THR   PRO   [ALA]  [ALA]  ASN   ASN   ARG   ALA   ARG   A
G C A C A C C A G C A G C A A A C A A T C G A G C C A G A A
C G T G T G G T C G T C G T T T G T T A G C T C G G T C T T
                  310                 320                 330

RG   GLU   LEU   PRO   ARG   PHE   MET   ASN   TYR   THR   L
G A G A A C T A C C A A G G T T T A T G A A T T A T A C A C
C T C T T G A T G G T T C C A A A T A C T T A A T A T G T G
                  340                 350                 360

EU   ASN   ASN  [THR]  LYS   LYS   THR   ASN   VAL   THR   L
T C A A C A A T A C C A A A A A A A C C A A T G T A A C A T
A G T T G T T A T G G T T T T T T T G G T T A C A T T G T A
                  370                 380                 390

                                                F2-F1 CLEAVAGE
EU   SER   LYS   LYS   ARG   LYS   ARG   ARG  ↓PHE   LEU   G
T A A G C A A G A A A A G G A A A A G A A G A T T T C T T G
A T T C G T T C T T T T C C T T T T C T T C T A A A G A A C
                  400                 410                 420

LY   PHE   LEU   LEU   GLY   VAL   GLY   SER   ALA   ILE   A
G T T T T T T G T T A G G T G T T G G A T C T G C A A T C G
C A A A A A A C A A T C C A C A A C C T A G A C G T T A G C
                  430                 440                 450

LA   SER   GLY  [ILE]  ALA   VAL   SER   LYS   VAL   LEU   H
C C A G T G G C A T T G C T G T A T C T A A G G T C C T G C
G G T C A C C G T A A C G A C A T A G A T T C C A G G A C G
                  460                 470                 480

IS   LEU   GLU   GLY   GLU   VAL   ASN   LYS   ILE   LYS   S
A C T T A G A A G G A G A A G T G A A C A A G A T C A A A A
T G A A T C T T C C T C T T C A C T T G T T C T A G T T T T
                  490                 500                 510

ER   ALA   LEU   LEU   SER   THR   ASN   LYS   ALA   VAL   V
G T G C T C T A C T A T C C A C A A A C A A G G C C G T A G
C A C G A G A T G A T A G G T G T T T G T T C C G G C A T C
                  520                 530                 540

AL   SER   LEU   SER   ASN   GLY   VAL   SER   VAL   LEU   T
T C A G C T T A T C A A A T G G A G T T A G T G T C T T A A
A G T C G A A T A G T T T A C C T C A A T C A C A G A A T T
                  550                 560                 570

HR   SER   LYS   VAL   LEU   ASP   LEU   LYS   ASN   TYR   I
C C A G C A A A G T G T T A G A C C T C A A A A A C T A T A
G G T C G T T T C A C A A T C T G G A G T T T T T G A T A T
                  580                 590                 600
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LE   ASP   LYS   GLN   LEU   LEU   PRO   ILE   VAL   ASN   L
TAGATAAACAATTGTTACCTATTGTGAATA
ATCTATTTGTTAACAATGGATAACACTTAT
                610                   620                   630

YS   GLN   SER   CYS  [ARG]  ILE   SER   ASN   ILE   GLU   T
AGCAAAGCTGCAGAATATCAAATATAGAAA
TCGTTTCGACGTCTTATAGTTTATATCTTT
                640                   650                   660

HR   VAL   ILE   GLU   PHE   GLN   GLN   LYS   ASN   ASN   A
CTGTGATAGAGTTCCAACAAAAGAACAACA
GACACTATCTCAAGGTTGTTTCTTGTTGT
                670                   680                   690

RG   LEU   LEU   GLU   ILE   THR   ARG   GLU   PHE   SER   V
GACTACTAGAGATTACCAGGGAATTTAGTG
CTGATGATCTCTAATGGTCCCTTAAATCAC
                700                   710                   720

AL   ASN   ALA   GLY   VAL   THR   THR   PRO   VAL   SER   T
TTAATGCAGGTGTAACTACACCTGTAAGCA
AATTACGTCCACATTGATGTGGACATTCGT
                730                   740                   750

HR   TYR   MET   LEU   THR   ASN   SER   GLU   LEU   LEU   S
CTTACATGTTAACTAATAGTGAATTATTGT
GAATGTACAATTGATTATCACTTAATAACA
                760                   770                   780

ER   LEU   ILE   ASN   ASP   MET   PRO   ILE   THR   ASN   A
CATTAATCAATGATATGCCTATAACAAATG
GTAATTAGTTACTATACGGATATTGTTTAC
                790                   800                   810

SP   GLN   LYS   LYS   LEU   MET   SER   ASN   ASN   VAL   G
ATCAGAAAAAAGTTAATGTCCAACAATGTTC
TAGTCTTTTTCAATTACAGGTTGTTACAAG
                820                   830                   840

LN   ILE   VAL   ARG   GLN   GLN   SER   TYR   SER   ILE   M
AAATAGTTAGACAGCAAAGTTACTCTATCA
TTTATCAATCTGTCGTTTCAATGAGATAGT
                850                   860                   870

ET   SER   ILE   ILE   LYS   GLU   GLU   VAL   LEU   ALA   T
TGTCCATAATAAAAGAGGAAGTCTTAGCAT
ACAGGTATTATTTTCTCCTTCAGAATCGTA
                880                   890                   900
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YR   VAL   VAL   GLN   LEU   PRO   LEU   TYR   GLY   VAL   I
A T G T A G T A C A A T T A C C A C T A T A T G G T G T G A
T A C A T C A T G T T A A T G G T G A T A T A C C A C A C T
                  910                 920                 930

LE   ASP   THR   PRO   CYS   TRP   LYS   LEU   HIS   THR   S
T A G A T A C A C C T T G T T G G A A A T T A C A C A C A T
A T C T A T G T G G A A C A A C C T T T A A T G T G T G T A
                  940                 950                 960

ER   PRO   LEU   CYS   THR   THR   ASN   THR   LYS   GLU   G
C C C C T C T A T G T A C A A C C A A C A C A A A A G A A G
G G G G A G A T A C A T G T T G G T T G T G T T T T C T T C
                  970                 980                 990

LY   SER   ASN   ILE   CYS   LEU   THR   ARG   THR   ASP   A
G G T C A A A C A T C T G T T T A A C A A G A A C T G A C A
C C A G T T T G T A G A C A A A T T G T T C T T G A C T G T
                 1000                1010                1020

RG   GLY   TRP   TYR   CYS   ASP   ASN   ALA   GLY   SER   V
G A G G A T G G T A C T G T G A C A A T G C A G G A T C A G
C T C C T A C C A T G A C A C T G T T A C G T C C T A G T C
                 1030                1040                1050

AL   SER   PHE   PHE   PRO   GLN   ALA   GLU   THR   CYS   L
T A T C T T T C T T C C C A C A A G C T G A A A C A T G T A
A T A G A A A G A A G G G T G T T C G A C T T T G T A C A T
                 1060                1070                1080

YS   VAL   GLN   SER   ASN   ARG   VAL   PHE   CYS   ASP   T
A A G T T C A A T C G A A T C G A G T A T T T T G T G A C A
T T C A A G T T A G C T T A G C T C A T A A A A C A C T G T
                 1090                1100                1110

HR   MET   ASN   SER   LEU   THR   LEU   PRO   SER   GLU   V
C A A T G A A C A G T T T A A C A T T A C C A A G T G A A G
G T T A C T T G T C A A A T T G T A A T G G T T C A C T T C
                 1120                1130                1140

AL   ASN   LEU   CYS   ASN   VAL   ASP   ILE   PHE   ASN   P
T A A A T C T C T G C A A T G T T G A C A T A T T C A A T C
A T T T A G A G A C G T T A C A A C T G T A T A A G T T A G
                 1150                1160                1170

RO   LYS   TYR   ASP   CYS   LYS   ILE   MET   THR   SER   L
C C A A A T A T G A T T G T A A A A T T A T G A C T T C A A
G G T T T A T A C T A A C A T T T T A A T A C T G A A G T T
                 1180                1190                1200
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YS   THR   ASP   VAL   SER   SER   SER   VAL   ILE   THR   S
A A A C A G A T G T A A G C A G C T C C G T T A T C A C A T
T T T G T C T A C A T T C G T C G A G G C A A T A G T G T A
                1210                1220                1230

ER   LEU   GLY   ALA   ILE   VAL   SER   CYS   TYR   GLY   L
C T C T A G G A G C C A T T G T G T C A T G C T A T G G C A
G A G A T C C T C G G T A A C A C A G T A C G A T A C C G T
                1240                1250                1260

YS   THR   LYS   CYS   THR   ALA   SER   ASN   LYS   ASN   A
A A A C T A A A T G T A C A G C A T C C A A T A A A A A T C
T T T G A T T T A C A T G T C G T A G G T T A T T T T T A G
                1270                1280                1290

RG   GLY   ILE   ILE   LYS   THR   PHE   SER   ASN   GLY   C
G T G G A A T C A T A A A G A C A T T T T C T A A C G G G T
C A C C T T A G T A T T T C T G T A A A A G A T T G C C C A
                1300                1310                1320

YS   ASP   TYR   VAL   SER   ASN   LYS   GLY   VAL   ASP   T
G T G A T T A T G T A T C A A A T A A A G G G G T G G A C A
C A C T A A T A C A T A G T T T A T T T C C C C A C C T G T
                1330                1340                1350

HR   VAL   SER   VAL   GLY   ASN   THR   LEU   TYR   TYR   V
C T G T G T C T G T A G G T A A C A C A T T A T A T T A T G
G A C A C A G A C A T C C A T T G T G T A A T A T A A T A C
                1360                1370                1380

AL   ASN   LYS   GLN   GLU   GLY   LYS   SER   LEU   TYR   V
T A A A T A A G C A A G A A G G C A A A A A G T C T C T A T G
A T T T A T T C G T T C T T C C G T T T T C A G A G A T A C
                1390                1400                1410

AL   LYS   GLY   GLU   PRO   ILE   ILE   ASN   PHE   TYR   A
T A A A A A G G T G A A C C A A T A A T A A A T T T C T A T G
A T T T T C C A C T T G G T T A T T A T T T A A A G A T A C
                1420                1430                1440

SP   PRO   LEU   VAL   PHE   PRO   SER   ASP   GLU   PHE   A
A C C C A T T A G T A T T C C C C T C T G A T G A A T T T G
T G G G T A A T C A T A A G G G G A G A C T A C T T A A A C
                1450                1460                1470

SP   ALA   SER   ILE   SER   GLN   VAL   ASN   GLU   LYS   I
A T G C A T C A A T A T C T C A A G T C A A T G A G A A G A
T A C G T A G T T A T A G A G T T C A G T T A C T C T T C T
                1480                1490                1500
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LE   ASN   GLN   SER   LEU   ALA   PHE   ILE   ARG   LYS   S
T T A A C C A G A G T T A G C A T T T A T T C G T A A A T
A A T T G G T C T C A A A T C G T A A A T A A G C A T T T A
                1510                  1520                  1530

ER   ASP   GLU   LEU   LEU   HIS   ASN   VAL   ASN   ALA   G
C C G A T G A A T T A T T A C A T A A T G T A A A T G C T G
G G C T A C T T A A T A A T G T A T T A C A T T T A C G A C
                1540                  1550                  1560
                                      <-----------------------

LY   LYS   SER   THR   THR   ASN   ILE   MET   ILE   THR   T
G T A A A T C A A C C A C A A A T A T C A T G A T A A C T A
C A T T T A G T T G G T G T T T A T A G T A C T A T T G A T
                1570                  1580                  1590
                                  TM
HR   ILE   ILE   ILE   VAL   ILE   ILE   VAL   ILE   LEU   L
C T A T A A T T A T A G T G A T T A T A G T A A T A T T G T
G A T A T T A A T A T C A C T A A T A T C A T T A T A A C A
                1600                  1610                  1620

EU   SER   LEU   ILE   ALA   VAL   GLY   LEU   LEU   LEU   T
T A T C A T T A A T T G C T G T T G G A C T G C T C C T A T
A T A G T A A T T A A C G A C A A C C T G A C G A G G A T A
                1630                  1640                  1650
------>
YR   CYS   LYS   ALA   ARG   SER   THR   PRO   VAL   THR   L
A C T G T A A G G C C A G A A G C A C A C C A G T C A C A C
T G A C A T T C C G G T C T T C G T G T G G T C A G T G T G
                1660                  1670                  1680

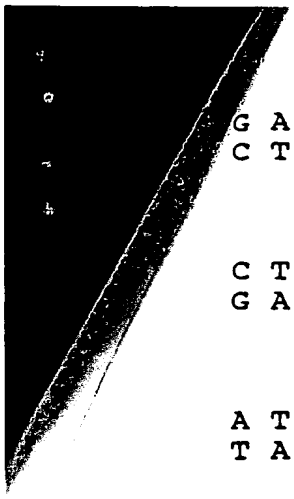
EU   SER   LYS   ASP   GLN   LEU   SER   GLY   ILE   ASN   A
T A A G C A A G G A T C A A C T G A G T G G T A T A A A T A
A T T C G T T C C T A G T T G A C T C A C C A T A T T T A T
                1690                  1700                  1710

SN   ILE   ALA   PHE   SER   ASN   * * *

A T A T T G C A T T T A G T A A C T G A A T A A A A A T A G
T A T A A C G T A A A T C A T T G A C T T A T T T T T A T C
                1720                  1730                  1740


C A C C T A A T C A T G T T C T T A C A A T G G T T T A C T
G T G G A T T A G T A C A A G A A T G T T A C C A A A T G A
                1750                  1760                  1770


A T C T G C T C A T A G A C A A C C C A T C T A T C A T T G
T A G A C G A G T A T C T G T T G G G T A G A T A G T A A C
                1780                  1790                  1800
```



```
G A T T T T C T T A A A A T C T G A A C T T C A T C G A A A
C T A A A A G A A T T T T A G A C T T G A A G T A G C T T T
                1810                1820                1830

C T C T T A T C T A T A A A C C A T C T C A C T T A C A C T
G A G A A T A G A T A T T T G G T A G A G T G A A T G T G A
                1840                1850                1860

A T T T
T A A A
```

Figure 5: Nucleotide and amino acid sequences of the RSV F gene. The cDNA sequence is shown in the plus (mRNA) strand sense in the 5' to 3' direction. The signal peptide (SP) and the transmembrane (TM) anchor domain are underlined. The predicted F2-F1 cleavage site is indicated by the arrow (↓). Amino acids differing from the published coding sequence of the RSV F gene are boxed.

FIGURE 6: RESTRICTION MAP OF THE RSV F GENE

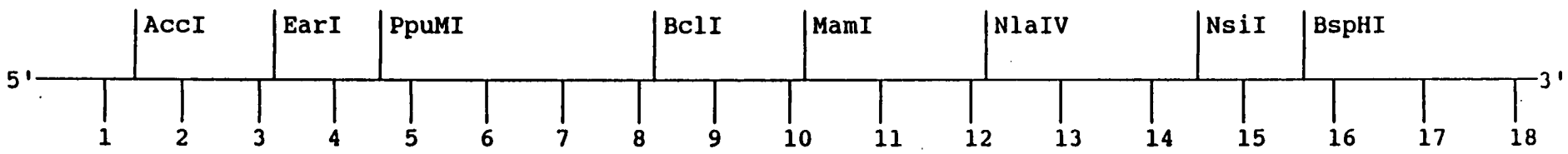


FIGURE 7: SEQUENCE OF OLIGONUCLEOTIDE CASSETTES

Fig. 7A

```
   BsrI                          BamHI

5' --ATCAATCAAAGGTCCTGTGATAATAG----  3'
   CGTAGTTAGTTTCCAGGACACTATTATCCTAG
```

Fig. 7B

```
   BspHI            BamHI

5' CATGACTTGATAATGAG---- 3'
   ----TGAACTATTACTCCTAG
```

Fig. 7C

```
   EcoRI                                                                PpuMI

5' AATTCATGGAGTTGCTAATCCTCAAAGCAAATGCAATTACCACAATCCTCACTGCAGTCACATTTTGTTTTGCTTCTGGTTCTAAG--- 3'
   ----GTACCTCAACGATTAGGAGTTTCGTTTACGTTAATGGTGTTAGGAGTGACGTCAGTGTAAAACAAAACGAAGACCAAGATTCCAG
```

Fig. 7D

```
   BsrI                          BamHI

5' ACTGGCATCAATCTAGCACTACATGAG---- 3'
   ----CGTAGTTAGATCGTGATGTACTCCTAG
```

Fig. 7E

```
   EcoRI

5' AATTCATGCCAACTTTAATACTGCTAATTATTACAACAATGATTATGGCATCTTCCTGCCAAATAGATATCACAAAACTACAGCATGTAGGTGTATTGGTCAACAGTCCC
   ----GTACGGTTGAAATTATGACGATTAATAATGTTGTTACTAATACCGTAGAAGGACGGTTTATCTATAGTGTTTTGATGTCGTACATCCACATAACCAGTTGTCAGGG

                                                                        BstBI

                                                    AAAGGGATGAAGATATCACAAAACTT----
                                                    TTTCCCTACTTCTATAGTGTTTTGAAGCTT
```

FIGURE 8:

SEQUENCE OF OLIGONUCLEOTIDE CASSETTE USED TO RESTORE NATIVE POLYHEDRIN PROMOTER IN THE pAc 610 VECTOR

EcoRV EcoRI

ATCATGGAGATAATTAAAATGATAACCATCTCGCAAATAAATAAGTATTTTACTGTTTTCGTAACAGTTTTGTAATAAAAAAACCTATAAATAG----

TAGTACCTCTATTAATTTTACTATTGGTAGAGCGTTTATTTATTCATAAAATGACAAAAGCATTGTCAAAACATTATTTTTTTGGATATTTATCTTAA

FIGURE 9: IMMUNOBLOTS OF CELL LYSATES FROM Sf9 CELLS INFECTED WITH RECOMBINANT BACULOVIRUSES

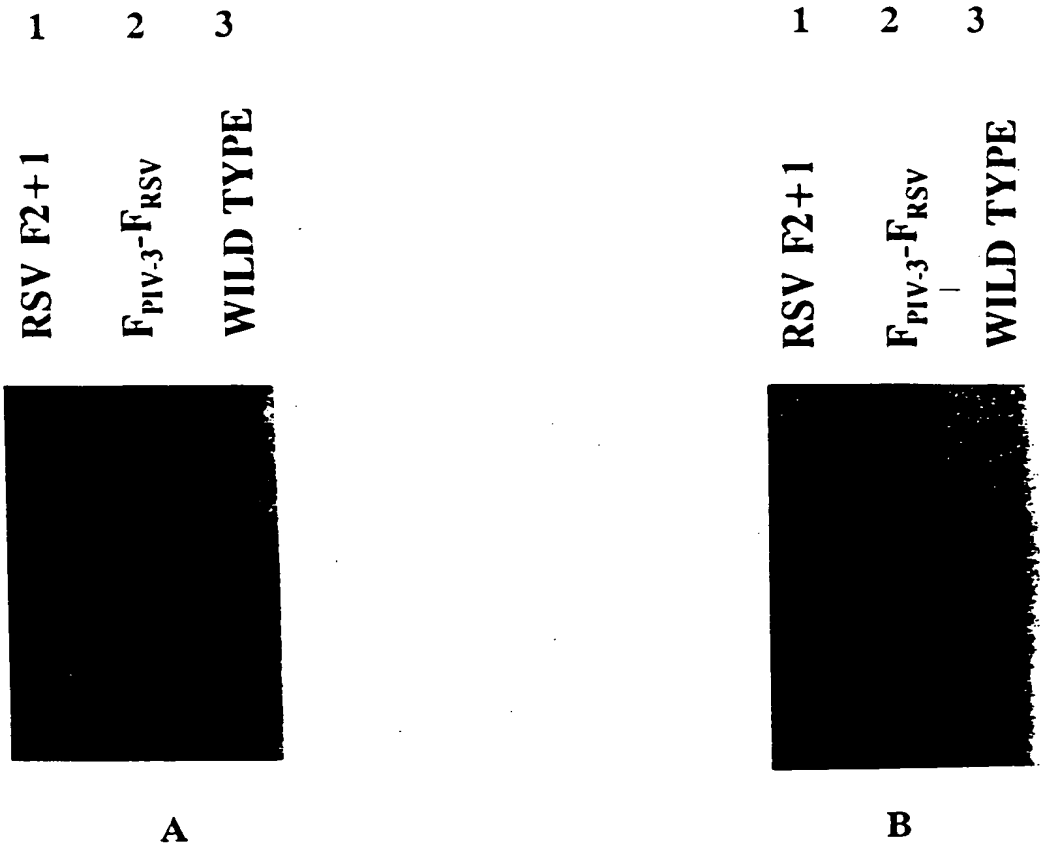


Figure 9: Immunoblots of cell lysates from Sf9 cells infected with recombinant baculoviruses containing the truncated RSV F gene (Lane 1), the chimeric F_{PIV-3}-F_{RSV} gene (Lane 2) or infected with wild type virus (Lane 3) reacted with anti-F RSV Mab (panel A) and anti-F1 PIV-3 antiserum (panel B).

FIGURE 10: SEQUENCE OF OLIGONUCLEOTIDE CASSETTE